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MOLECULAR BIOLOGY OF THE CELL

Bruce Alberts • Dennis Bray
Julian Lewis • Martin Raff • Keith Roberts
James D. Watson



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"Long ago it became evident that the key to every biological problem must finally be sought in the cell, for every living organism is, or at sometime has been, a cell."

Edmund B. Wilson
The Cell in Development and Heredity
3rd edition, 1925, Macmillan, Inc.

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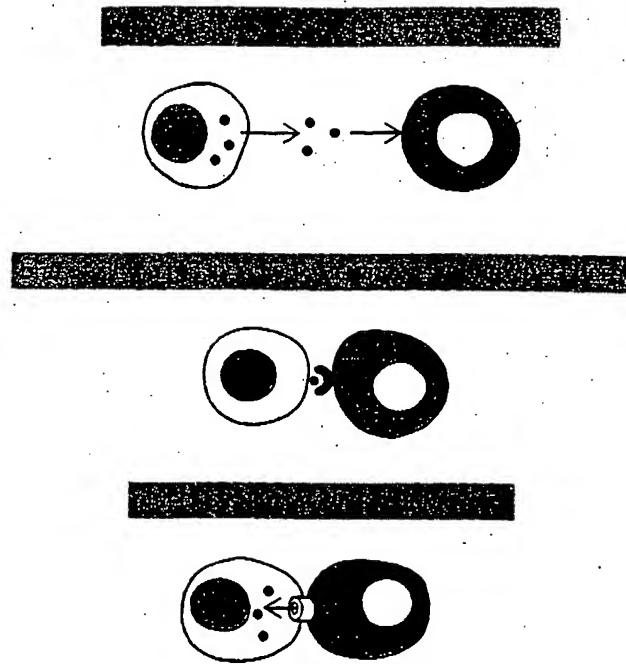


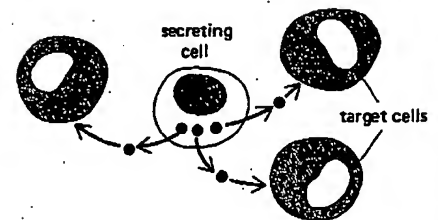
Figure 13-1 Schematic diagram showing three different ways in which cells are thought to communicate with each other.

Three Different Strategies of Chemical Signaling: Local Chemical Mediators, Hormones, and Neurotransmitters

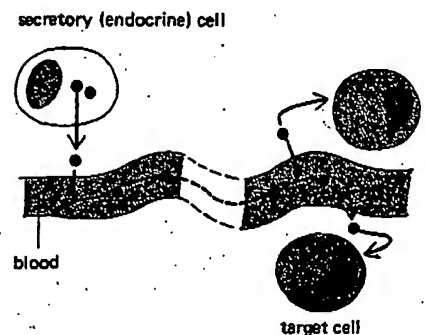
Chemical signaling operates in three different ways: (1) most cells in the body secrete one or more chemical signals that function as **local chemical mediators** because they are so rapidly taken up or destroyed that they act only on cells in the immediate environment; (2) specialized *endocrine cells* secrete **hormones** that travel through the bloodstream to influence target cells widely distributed in the body; and (3) nerve cells form specialized junctions (*chemical synapses*) with the target cells they influence and secrete very short-range chemical mediators called **neurotransmitters**, which act only on the adjoining target cell (Figure 13-2).

Endocrine cells and nerve cells are highly specialized for chemical signaling and work together to coordinate the diverse activities of the billions of cells in a higher animal. Nerve cells transmit information much more rapidly than endocrine cells because they do not depend on diffusion or blood flow to convey information over long distances; instead, electrical impulses carry the signal rapidly along nerve processes. Only at the nerve terminals, when a neurotransmitter is released, are the electrical impulses converted into chemical signals; the neurotransmitter has to diffuse only a microscopic distance to the target cell, a process that takes less than a millisecond (Figure 13-2). While hormones are greatly diluted in the bloodstream and therefore must be able to act at very low concentrations (typically $<10^{-8}$ M), neurotransmitters are diluted much less and can achieve high concentrations in the region of the target cell. For example, the concentration of the neurotransmitter acetylcholine in the synaptic cleft of an active neuromuscular junction is about 5×10^{-4} M. In other respects, however, the mechanisms of chemical signaling by hormones and neurotransmitters are generally similar, and many of the signaling molecules that are used by endocrine cells are also used by nerve cells (neurons).

LOCAL CHEMICAL MEDIATOR



HORMONE



NEUROTRANSMITTER

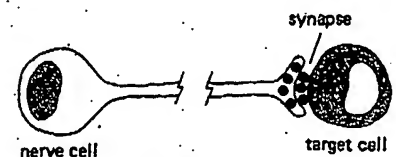
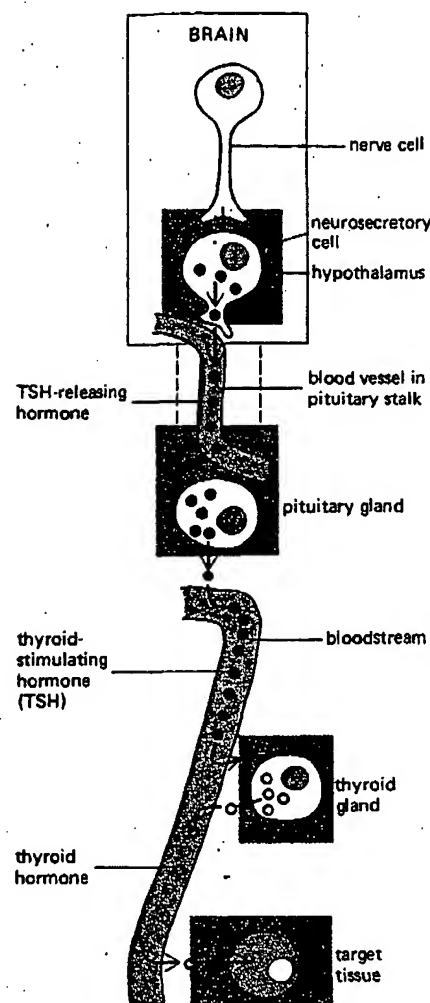


Figure 13-2 Three different classes of extracellular signaling molecules.

Figure 13-3 Schematic illustration of the indirect manner in which thyroid hormone secretion is regulated by the nervous system. When stimulated by nerve cells in higher centers of the brain, specific neurosecretory cells in the hypothalamus secrete into blood vessels of the pituitary stalk TSH-releasing hormone, which stimulates the release of TSH (thyroid-stimulating hormone) by specific cells in the pituitary gland. TSH in turn stimulates the cells in the thyroid gland to synthesize and secrete thyroid hormone. Thyroid hormone then stimulates a variety of metabolic processes in most cells in the body. Not illustrated in this figure is the fact that the secretion of both TSH-releasing hormone and TSH are suppressed by increased concentrations of thyroid hormone in the blood. This *feedback inhibition* prevents the blood levels of thyroid hormone from rising too high.



The Hypothalamus Is the Main Regulator of the Endocrine System¹

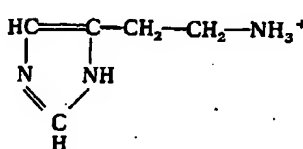
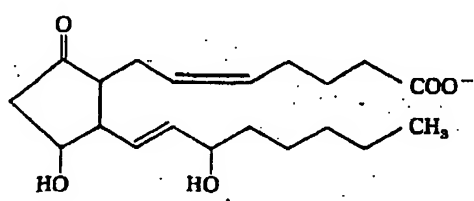
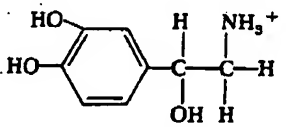
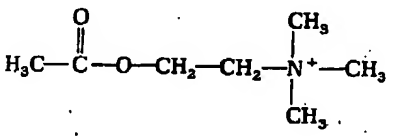
The endocrine system and nervous system are physically and functionally linked by a specific region of the brain called the **hypothalamus**. The hypothalamus lies immediately above the pituitary gland, to which it is connected by a hypothalamic extension called the *pituitary stalk*. The bridging function of the hypothalamus is mediated by cells that have properties of both nerve cells and endocrine cells: they have nerve processes that carry electrical impulses but release their signaling molecules into the blood; for this reason they are called *neurosecretory cells*. Each of the hypothalamic neurosecretory cells can be stimulated by other nerve cells in higher regions of the brain to secrete a specific peptide hormone into the blood vessels of the pituitary stalk; the hormone then specifically stimulates or suppresses the secretion of a second hormone from the pituitary. Many of the pituitary hormones regulated by the hypothalamus in this way stimulate another endocrine gland to secrete a third hormone into the blood. Consequently, the hypothalamus serves as the main regulator of the endocrine system. Figure 13-3 illustrates how this hierarchy works in the regulation of the secretion of *thyroid hormone*.

Selected examples of local chemical mediators, neurotransmitters, and hormones are given in Table 13-1, together with their sites of origin, structures, and principal actions. For the most part, the examples chosen are discussed elsewhere in this book. It can be seen that these signaling molecules are as varied in structure as they are in function. They include small peptides, larger proteins and glycoproteins, amino acids and related compounds, steroids (molecules derived from cholesterol and closely related in structure), and fatty acid derivatives.

Different Cells Respond in Different Ways to the Same Chemical Signal²

The ability of a cell to respond to a particular extracellular signaling molecule—depends on its having specific proteins, called **receptors**, that bind the signaling molecule. Many signaling molecules act at very low concentration (typically $\leq 10^{-8}$ M), and their complementary receptors usually bind them with high affinity (affinity constant $K \geq 10^8$ liters per mole; see p. 97). In mature

Table 13-1 Some Examples of Signaling Molecules

Local Chemical Mediators	Site of Origin	Structure	Major Effects
<i>Proteins</i>			
Nerve growth factor	all tissues innervated by sympathetic nerves	2 identical chains of 118 amino acids	survival and growth of sensory and sympathetic neurons
<i>Small Peptides</i>			
Eosinophil chemotactic factor	mast cells	4 amino acids	chemotactic signal for a special type of white blood cell (eosinophilic leukocytes)
<i>Amino Acid Derivatives</i>			
Histamine	mast cells		causes blood vessels to dilate and become leaky
<i>Fatty Acid Derivatives</i>			
Prostaglandin E ₂	many different cell types		contraction of smooth muscle
<i>Neurotransmitters*</i>			
<i>Amino Acids and Related Compounds</i>			
Glycine	nerve terminals	$^+ \text{H}_3\text{N}-\text{CH}_2-\text{COO}^-$	inhibitory transmitter in central nervous system
Norepinephrine	nerve terminals		excitatory and inhibitory transmitter in central and peripheral nervous system
γ-Aminobutyric acid (GABA)	nerve terminals	$^+ \text{H}_3\text{N}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{COO}^-$	inhibitory transmitter in central nervous system
Acetylcholine	nerve terminals		excitatory transmitter at neuromuscular junction; excitatory and inhibitory transmitter in central and peripheral nervous system
<i>Small Peptides</i>			
Enkephalin	nerve terminals	5 amino acids	morphine-like action (inhibits pain pathways in central nervous system)

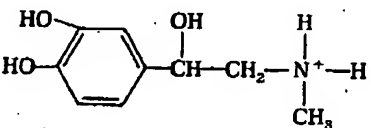
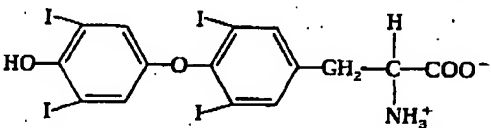
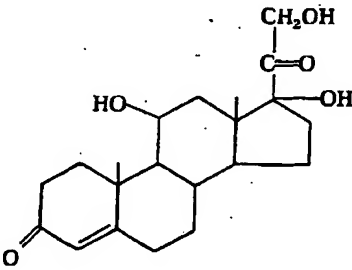
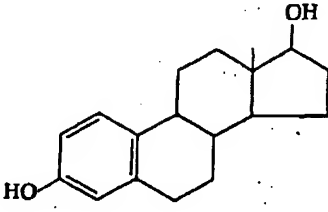
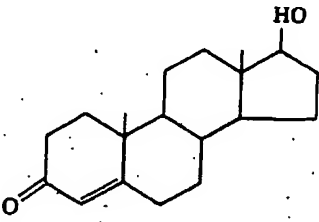
*Some of these molecules may function as local chemical mediators in the nervous system rather than strictly as neurotransmitters (see p. 726). Excitatory neurotransmitters stimulate the postsynaptic cell, while inhibitory neurotransmitters suppress the postsynaptic cell.

Table 13-1 Continued

Hormones	Site of Origin	Structure	Major Effects
<i>Proteins and Glycoproteins</i>			
Insulin	beta cells of pancreas	protein α -chain = 21 amino acids β -chain = 30 amino acids	utilization of carbohydrate (including uptake of glucose into cells); stimulation of protein synthesis; stimulation of lipid synthesis in fat cells
Somatotropin (growth hormone)	anterior pituitary	protein 191 amino acids	stimulation of liver to produce somatomedins, which in turn cause growth of muscle and bone
Somatomedins	liver	proteins	growth of bone and muscle; influences metabolism of Ca^{2+} , phosphate, carbohydrate, and lipid
Adrenocorticotrophic hormone (ACTH)	anterior pituitary	protein 39 amino acids	stimulation of adrenal cortex to produce cortisol; fatty acid release from fat cells
Parathormone	parathyroid	protein 84 amino acids	increase in bone resorption, thereby increasing blood Ca^{2+} and phosphate; increase in resorption of Ca^{2+} and Mg^{2+} and decrease in resorption of phosphate in kidney tubules
Follicle-stimulating hormone (FSH)	anterior pituitary	glycoprotein α -chain = 92 amino acids β -chain = 118 amino acids	stimulation of ovarian follicles to grow and secrete estradiol; stimulation of spermatogenesis in testis
Luteinizing hormone (LH)	anterior pituitary	glycoprotein α -chain = 92 amino acids β -chain = 115 amino acids	stimulation of oocyte maturation and ovulation and progesterone secretion from ovary; stimulation of testis to produce testosterone
Epidermal growth factor	unknown	53 amino acids	stimulation of epidermal and other cells to divide
Thyroid-stimulating hormone (TSH)	anterior pituitary	glycoprotein α -chain = 92 amino acids β -chain = 112 amino acids	stimulation of thyroid to produce thyroxine; fatty acid release from fat cells
<i>Small Peptides</i>			
TSH-releasing factor	hypothalamus	3 amino acids	stimulation of anterior pituitary to secrete thyroid-stimulating hormone (TSH)

(Continued)

Table 13-1 Continued

Hormones	Site of Origin	Structure	Major Effects
LH-releasing factor	hypothalamus	10 amino acids	stimulation of anterior pituitary to secrete luteinizing hormone (LH)
Vasopressin	posterior pituitary	9 amino acids	elevation of blood pressure by constriction of small blood vessels; increase in water resorption in kidney tubules
Somatostatin	hypothalamus	14 amino acids	inhibition of somatotropin release from anterior pituitary
Amino Acid Derivatives Epinephrine	adrenal medulla		increase in blood pressure and heart rate; increase in glycogenolysis in liver and muscle; fatty acid release from fat cells
Thyroxine	thyroid		increase in metabolic activity in most cells
Steroids Cortisol	adrenal cortex		affect on metabolism of proteins, carbohydrates and lipids; suppression of inflammatory reactions
Estradiol	ovary, placenta		development and maintenance of secondary female sex characteristics; maturation and cyclic function of accessory sex organs; development of duct system in mammary glands
Testosterone	testis		development and maintenance of secondary male sex characteristics; maturation and normal function of accessory sex organs

animals most cells are specialized to perform one primary function, and they contain a characteristic array of receptors that allows them to respond to each of the different chemical signals that initiate or modulate that function.

Most chemical signals ultimately influence target cells either by altering the properties or rates of synthesis of existing proteins or by initiating the synthesis of new ones. In different target cells the same signaling molecule often affects different proteins and therefore has different effects. For example, *acetylcholine* stimulates the contraction of skeletal muscle cells, but it decreases the rate and force of contraction in heart muscle cells. In this particular case the acetylcholine receptor proteins on skeletal muscle cells are different from those on heart muscle cells. But receptor differences are not always the explanation. In many cases the same signaling molecules bind to identical receptor proteins and yet produce very different responses in different target cells. This indicates that target cells are programmed in two ways: (1) they are equipped with a distinctive set of receptors for responding to a complementary set of chemical signals, and (2) they are programmed to respond to each signal in their own characteristic way (Figure 13-4).

Some Cellular Responses to Chemical Signals Are Rapid and Transient, While Others Are Slow and Long-lasting

When they coordinate the responses of cells to changes in an animal's environment, chemical signals generally induce rapid and transient responses. For example, an increase in blood glucose levels stimulates endocrine cells in the pancreas to secrete the protein hormone *insulin* into the blood. Within minutes the resulting increase in insulin concentration stimulates liver and muscle cells to take up more glucose, and blood glucose levels fall. Then the rate of insulin secretion and, consequently, the rate of glucose uptake by liver and muscle cells return to their previous levels. In this way a relatively constant blood glucose concentration is maintained. Neurotransmitters elicit even more rapid responses: skeletal muscle cells contract and relax again within milliseconds in response to acetylcholine released from nerve terminals at a neuromuscular junction.

Chemical signals also play an important part in animal development, often influencing when and how certain cells differentiate. These effects are usually slow in onset and long-lasting. For example, the steroid female sex hormone *estradiol* is secreted in large amounts by cells in the ovary around the time of puberty. Estradiol induces changes in a wide variety of cells in different parts of the body, changes that eventually lead to the development of secondary female characteristics, such as breast enlargement. While this effect is slowly reversed if estradiol secretion stops, some of the responses to steroid sex hormones during very early mammalian development are irreversible (see p. 722). Similarly, a tenfold increase in thyroid hormone levels in the blood of a tadpole induces all of the dramatic and irreversible changes that result in its transformation into a frog (Figure 13-5).

Figure 13-5 Various stages in the metamorphosis of a tadpole into a frog. All of these dramatic changes during metamorphosis are signaled by thyroid hormone. If the presumptive thyroid gland is removed from a developing embryo, the animal fails to undergo metamorphosis and continues to grow as a tadpole. If thyroid hormone is injected into such a giant tadpole, the tadpole transforms into a frog.

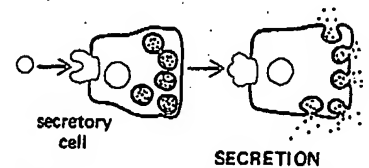
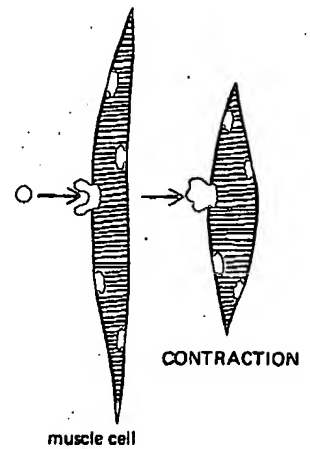
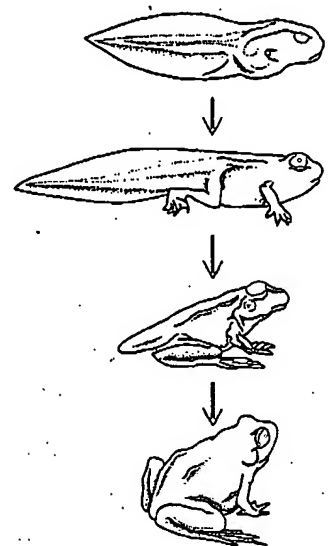


Figure 13-4 Schematic drawing showing how the same signaling molecule binding to identical receptors on two different target cells (muscle and secretory) can induce different responses. Each type of target cell is programmed to respond in a characteristic way to each specific signal.



Signaling Molecules Can Be Either Water-soluble or Lipid-soluble¹

All known neurotransmitters, as well as most hormones and local chemical mediators, are water-soluble. The main exceptions are the steroid and thyroid hormones, which are relatively water-insoluble and are made soluble for transport in the bloodstream by binding to specific carrier proteins. This difference in solubility gives rise to a fundamental difference in the mechanism by which the two classes of molecules influence target cells. Water-soluble molecules are too hydrophilic to pass directly through the lipid bilayer of a target-cell plasma membrane; instead they bind to specific receptor proteins on the cell surface. The steroid and thyroid hormones, on the other hand, are hydrophobic, and once released from their carrier proteins, they can pass easily through the plasma membrane of the target cells; these hormones bind to specific receptor proteins *inside* the cell (Figure 13-6).

Another important difference between these two classes of signaling molecules is the length of time that they persist in the bloodstream or tissue fluids. Water-soluble hormones are generally removed and/or broken down within minutes of entering the blood and the local chemical mediators and neurotransmitters are removed even faster, within seconds or milliseconds of entering the extracellular space. By contrast, steroid hormones persist in the blood for hours and thyroid hormone for days. Consequently, water-soluble signaling molecules usually mediate responses of short duration, while the water-insoluble molecules tend to mediate longer lasting responses.

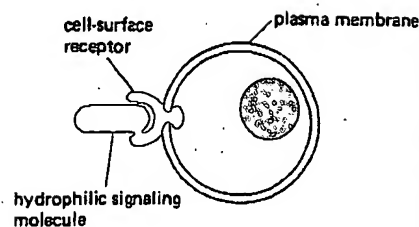
Local Chemical Mediators Are Rapidly Destroyed After They Are Secreted³

Many signaling molecules are secreted into the extracellular fluid and act only on cells in their immediate vicinity. These local chemical mediators are distinguished from hormones by the fact that they are so rapidly taken up by cells and/or destroyed that they generally do not enter the blood in significant amounts.

Some local mediators are secreted by cells specialized for that function. For example, *histamine* (a derivative of the amino acid histidine, see Table 13-1) is secreted mainly by mast cells. These cells, which are found in connective tissues throughout the body, store histamine in large secretory vesicles and release it rapidly by exocytosis when stimulated by injury, local infection, or certain immunological reactions. Histamine causes local blood vessels to dilate and become leaky, which facilitates the access of serum proteins (such as antibodies and components of the complement system, see Chapter 17) and phagocytic white blood cells to the sites of injury. Among the other mediators released by mast cells are two tetrapeptides that attract a class of white blood cells called *eosinophils* from the blood to the site of tetrapeptide release; eosinophils contain a variety of enzymes that help inactivate histamine and other chemical mediators released by mast cells.

While some local chemical mediators, like histamine, are secreted by specialized cells, others are of more widespread origin. The *prostaglandins*, a family of 20 carbon fatty acid derivatives, are an important example of such local mediators. Like other local mediators, prostaglandins are rapidly destroyed near the site of their synthesis by specific enzymes. Of the more than 16 different prostaglandins that belong to 9 classes (designated PGA, PGB, PGC, ... PGI), many are known to bind to different cell-surface receptors and to have different biological effects. Unlike most signaling molecules, they are not stored but are continuously released to the cell exterior. Prostaglandins are continuously synthesized in membranes from precursors that have been cleaved

HYDROPHILIC SIGNALS



HYDROPHOBIC SIGNALS

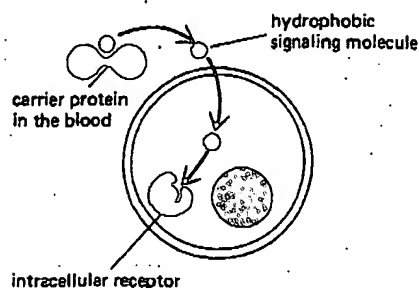


Figure 13-6 The differences in signaling mechanisms between hydrophilic and hydrophobic signaling molecules. Hydrophilic signaling molecules, being unable to cross the plasma membrane directly, bind to receptors on the surface of the target cell. Hydrophobic signaling molecules, being able to diffuse across the plasma membrane, bind to receptors inside the target cell. Because they are insoluble in aqueous solutions, hydrophobic signaling molecules are transported in the bloodstream bound to specific carrier proteins from which they dissociate prior to entering the target cell.

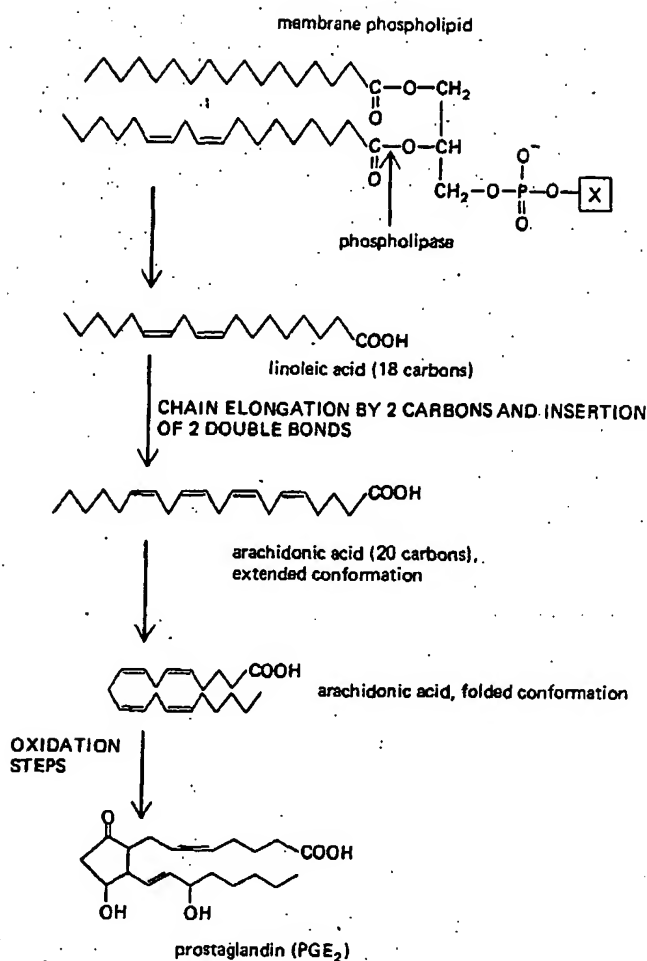


Figure 13-7 Synthesis of the prostaglandin PGE_2 . The subscript refers to the two carbon-carbon double bonds outside the ring of PGE_2 . Prostaglandins are continuously synthesized by most cells from fatty acid chains cleaved from membrane phospholipids.

from membrane phospholipids by phospholipases (Figure 13-7); they are also continuously degraded. However, when cells are activated by a change in their environment, many of them increase their rates of prostaglandin synthesis. The resulting increase in the local level of prostaglandins influences both the cell that makes the prostaglandins and its immediate neighbors.

A wide variety of biological activities have been ascribed to prostaglandins. They cause contraction of smooth muscle, aggregation of platelets, and inflammation. For example, certain prostaglandins produced in large amounts in the uterus at the time of childbirth seem to be important in stimulating the contraction of the uterine smooth muscle cells; these prostaglandins are now widely used as pharmacological agents to induce abortion. An important recent discovery has been that aspirin and some other anti-inflammatory agents probably work by inhibiting prostaglandin biosynthesis.

Not all local chemical mediators are rapidly destroyed after they have been secreted. Collagen and other macromolecules of the extracellular matrix can be considered as special types of local mediators. They are secreted by local cells and signal other local cells to alter their behavior. These molecules differ from other local chemical mediators in that they are insoluble and therefore do not diffuse from the region where they are synthesized. Consequently, unlike diffusible mediators, they do not have to be destroyed rapidly in order to prevent their effects from spreading.

Some Signaling Molecules Released by Nerve Terminals Probably Act as Local Chemical Mediators Rather Than as Neurotransmitters⁴

Neurotransmitters are either rapidly destroyed by specific enzymes in the synaptic cleft (as is the acetylcholine released at a neuromuscular junction) or rapidly retrieved by the nerve terminal that released them. Immediate removal serves two purposes: it confines the activity of the neurotransmitter to the postsynaptic cell, and it terminates the action of the transmitter molecule so that each signal is very brief and can be repeated almost immediately. This makes the signaling process extremely precise.

Nerve cells and endocrine cells direct their signaling molecules to target cells in very different ways. Each type of endocrine cell secretes a different hormone into the blood, and the specificity of the response depends entirely on which target cells have receptors for each hormone. Thus the endocrine system uses a large number of different hormones (and complementary receptors) to regulate the activities of many different target cells in specific ways. On the other hand, the speed, precision, and intricacy of signaling in the nervous system to a large extent depends on anatomical factors. Although target cells have specific receptors, most of the specificity of signaling depends on synaptic connections between nerve cells and their targets; neurotransmitters are released at synapses and influence only the adjacent postsynaptic cell (Figure 13-8). Some of these neurotransmitters are excitatory and stimulate the postsynaptic cell, while others are inhibitory and suppress the postsynaptic cell.

If all excitatory and inhibitory signaling in the central nervous system were focused on single cells in this way, there should be no need for more than a very small number of signaling molecules. But in fact more than 30 different signaling molecules have already been identified in the vertebrate

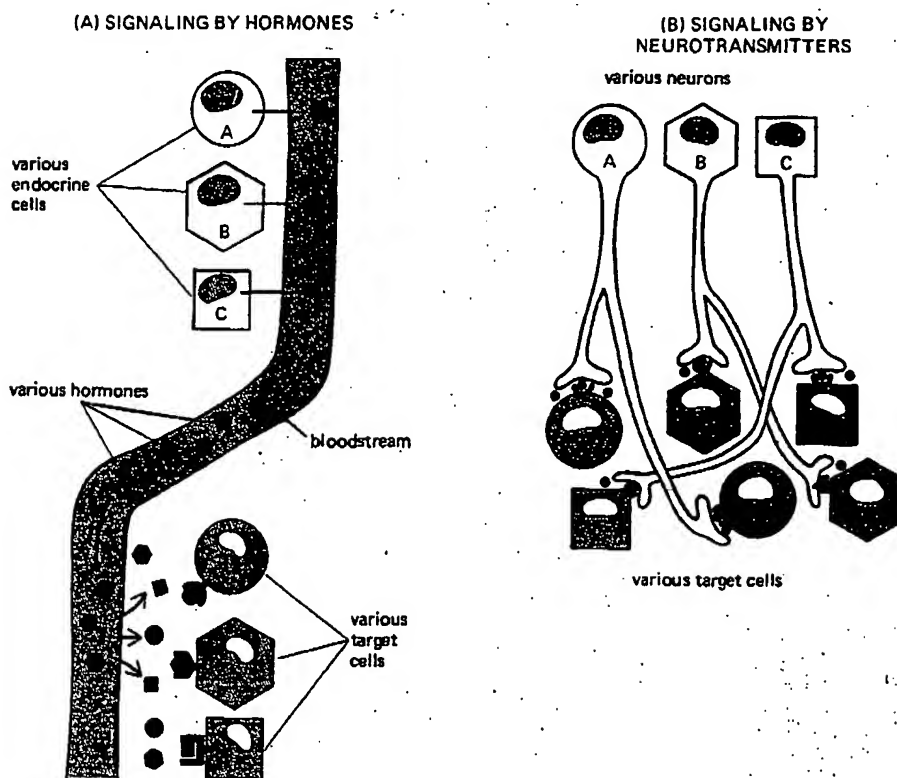


Figure 13-8 The contrast between cell signaling by means of hormones (A) and by means of neurotransmitters (B). Endocrine cells secrete many different hormones into the blood and signal specific target cells, which have receptors for binding specific hormones and thereby "pull" the appropriate hormones out of the extracellular fluid. By contrast, the specificity of signaling by many nerve cells arises from the contacts between their nerve processes and the specific target cells they signal: only a target cell having synaptic contact with a nerve cell is exposed to the neurotransmitter released from the nerve terminal. Whereas different endocrine cells must use different hormones in order to communicate with different target cells, different nerve cells can use the same neurotransmitter and still communicate in a specific manner.

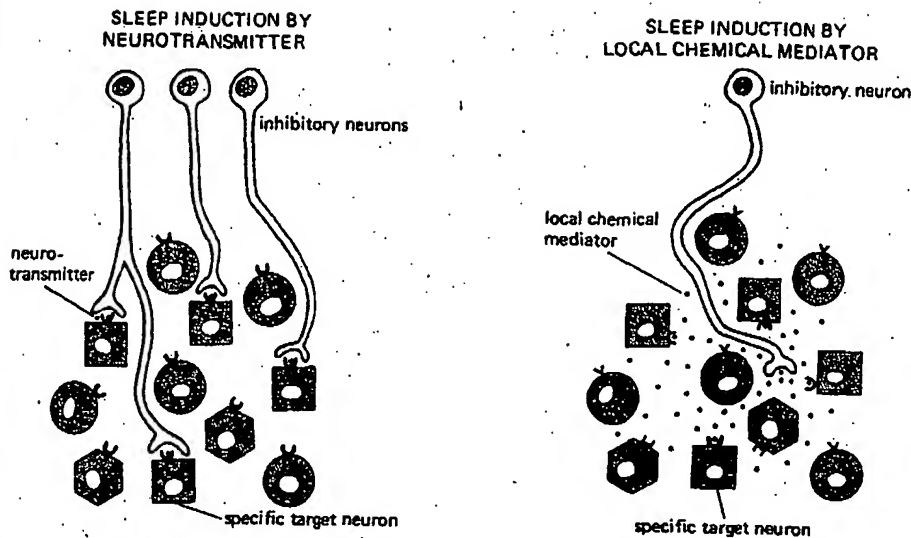


Figure 13-9 Two hypothetical schemes by which sleep might be induced if the prerequisite for sleep were the suppression of certain neurons (shown as squares). In principle, this could be achieved either with an inhibitory neurotransmitter released by neurons that synapse on all of the square cells or, more simply, by a local chemical mediator released from inhibitory nerve terminals in the general region where square cells are found. In the second case, the square cells would be the only ones suppressed because they alone possess receptors for the mediator.

brain, including acetylcholine; various amino acids (glycine, aspartic acid, glutamic acid, and γ -aminobutyric acid, or GABA), amino acid derivatives (norepinephrine, dopamine, serotonin, and histamine), and a large variety of peptides. This may mean that many signaling molecules function not as conventional neurotransmitters but as local chemical mediators (*neuromodulators*) that are released from nerve terminals and then diffuse locally to influence a large number of cells. Such signaling is not synaptic in the strict sense of the word (one terminal—one target cell), and so a large number of signaling molecules (and complementary receptors) are required to assure specificity, as is the case in the endocrine system.

Why might such a local mediator mechanism of neural signaling evolve? Suppose, for example, that sleep requires the suppression of a large number of specific nerve cells in a particular region of the brain. This suppression could be accomplished if specific nerve terminals containing an inhibitory neurotransmitter synapsed on all of these nerve cells. Alternatively, a relatively small number of nerve terminals might release an inhibitory local chemical mediator into the region; as long as all of the relevant nerve cells have receptors for such a "sleep substance," they would be suppressed (Figure 13-9). In fact, alert animals can be put to sleep by the injection (into their brain cavities) of cerebrospinal fluid from the brain cavities of animals that have been kept awake for many days.

Some Hormones and Local Chemical Mediators Act as Specific Growth Factors⁵

The rate of division of certain types of cells is regulated by chemical signals, some of which are conventional hormones. In mammals, estradiol causes breast epithelial cells to divide at puberty; in tadpoles, thyroxine induces particular muscle cells and cartilage cells to proliferate during metamorphosis, while at the same time it induces cells in the tail of the animal to self-destruct. The pituitary hormone *somatotropin* (also called *growth hormone*) indirectly stimulates cell division by inducing liver (and perhaps other) cells to secrete a number of protein hormones that cause certain cells to divide. These latter hormones, collectively called *somatomedins* because they mediate the effects of somatotropin, stimulate the growth and metabolism of muscle and cartilage cells: infants who produce too little somatotropin become dwarfs, while those who produce too much become giants.

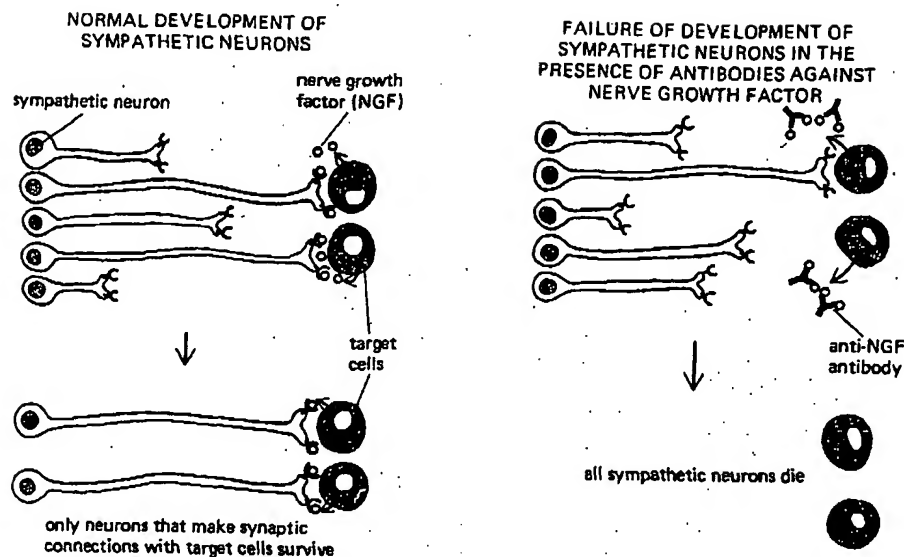


Figure 13-10 Schematic diagram showing that developing sympathetic neurons depend on nerve growth factor for survival. Treating developing animals with antibodies against nerve growth factor results in the death of sympathetic neurons. It is thought that nerve growth factor is released from target cells and that it binds to receptors present on the nerve terminals of the sympathetic neurons that make synapses on the target cells.

Other growth factors function as local chemical mediators rather than as hormones. The survival and growth of certain classes of nerve cells during development depend on **nerve growth factor (NGF)**, a protein dimer composed of two identical polypeptide chains 118 amino acids long, which is thought to be secreted by the target cells of these nerve cells. Three types of observation have demonstrated the importance of NGF for the survival of developing neurons of the sympathetic nervous system: (1) anti-NGF antibodies injected into newborn mice cause the selective death of sympathetic neurons (Figure 13-10); (2) many immature sympathetic neurons survive indefinitely in tissue culture in the absence of other cells if NGF is added to the culture medium; without NGF they die within a few days; (3) developing sympathetic neurons that fail to make synaptic connections with their target cells normally die but can be saved by injections of NGF.

Together these results suggest that many developing sympathetic neurons survive only if they are signaled by small amounts of NGF released by the target cells they innervate (Figure 13-10). Many central and peripheral nerve cells (including sympathetic neurons) produced during normal development are known to die within days of being formed; only those that manage to make synaptic contact with appropriate target cells seem to survive. It is likely that NGF is one of many neuronal survival factors and that different types of neurons require different specific factors produced by the target cells that they innervate in order to survive. The neuronal redundancy that occurs during neurogenesis presumably ensures that all target cells are innervated. NGF may also play a part in directing sympathetic nerve fibers to their appropriate target cells. When NGF is injected into the brain of a newborn mouse, it attracts sympathetic fibers to grow into the central nervous system,

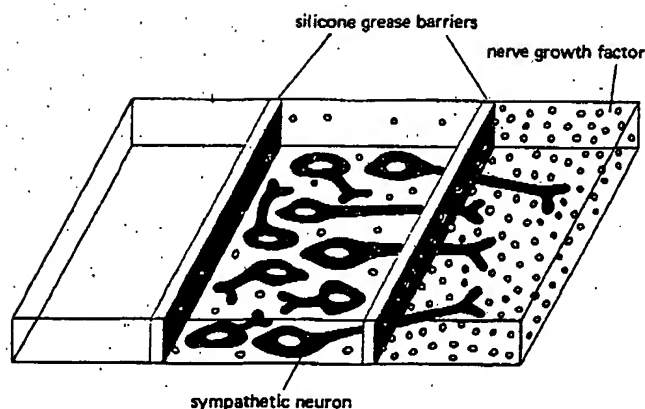


Figure 13-11 An experiment showing that nerve growth factor can influence the direction of nerve process outgrowth. When sympathetic neurons are placed in the central well of a three-chamber culture dish in which the chambers are separated by barriers of silicone grease, the cells are seen to extend nerve processes into the side chamber that contains nerve growth factor but not into the side chamber lacking the factor.

where normally they never go. An analogous phenomenon can be demonstrated *in vitro* if immature sympathetic neurons are placed in the central well of a three-chambered culture dish in which the chambers are separated by barriers of silicone grease, which is impermeable to NGF. Nerve fibers migrate beyond the silicone barriers into an adjacent chamber only if that chamber contains NGF (Figure 13-11).

Summary

Signaling molecules can be subdivided into three general classes according to their mechanism of delivery: (1) local chemical mediators are rapidly taken up or destroyed so that they act only on local cells; (2) hormones are carried in the blood to target cells throughout the body; and (3) neurotransmitters act only on the postsynaptic cell. Each cell type in the body contains a distinctive set of receptor proteins that enables it to bind and respond to a complementary set of signaling molecules in a preprogrammed and characteristic way.

The signaling molecules can also be classified according to their solubility in water. Hydrophobic signaling molecules, such as steroid hormones, pass through the plasma membrane and activate receptor proteins in the cell cytoplasm, while hydrophilic signaling molecules, including all neurotransmitters and the great majority of hormones and local chemical mediators, activate receptor proteins on the surface of the target cell.

Signaling Mediated by Intracellular Receptors: Mechanisms of Steroid Hormone Action

All steroid hormones are synthesized from cholesterol. Being relatively small (molecular weight ≈ 300) hydrophobic molecules, they cross the plasma membrane by simple diffusion. Once inside the target cell, each type of steroid hormone binds tightly but reversibly to a different receptor protein present in the cytoplasm. The binding of the hormone causes the receptor protein to undergo an allosteric change in its conformation that increases its ability to bind to DNA. Since the receptor proteins are able to migrate through the nuclear pores, their increased binding affinity for DNA causes the hormone-receptor complexes to accumulate in the cell nucleus (Figure 13-12).

The thyroid hormones are also small hydrophobic molecules that bind to intracellular receptors. They are thought to work like steroid hormones except that their receptors are concentrated in the nucleus even before hormone binding.

Figure 13-12 Schematic drawing of steroid hormone signaling. Steroid molecules diffuse across the plasma membrane of the target cell and bind to receptor proteins in the cytoplasm. The resulting hormone-receptor complexes then migrate into the nucleus where they bind to chromatin and regulate the transcription of specific genes.

Steroid-Hormone-Receptor Complexes Bind to Chromatin and Regulate the Transcription of Specific Genes⁶

A typical target cell contains about 10,000 steroid receptors, each of which will reversibly bind one molecule of a specific steroid hormone with high affinity (affinity constant $K = 10^8$ to 10^{10} liters per mole). When hormone levels are high, most receptors become complexed to hormone molecules and in this activated form bind to chromatin in the nucleus. As hormone levels fall, the equilibrium shifts so that hormone molecules dissociate from receptors and the freed receptors return to the cytoplasmic pool (Figure 13-13).

Some of the activated hormone receptors bound to chromatin regulate the transcription of specific genes. However, only a small number of genes in any target cell are directly influenced by steroid hormones. For example, 30 minutes after cultured rat liver cells are exposed to *cortisol*, only 6 of the 1000 proteins that can be distinguished by two-dimensional gel electrophoresis are increased in amount, and 1 is decreased. The synthesis rates of these proteins returns to normal once the hormone is removed. Assuming that about 10% of the cell's proteins can be detected on these gels, cortisol probably directly alters the transcription of only about 50 genes. Interestingly, the same hormone receptor is found to influence different sets of genes in different target cells (see following page).

Experiments performed *in vitro* have revealed that the binding of a specific steroid hormone induces an allosteric change in the steroid receptor (receptor activation) that greatly increases the affinity with which the receptor protein binds to DNA isolated from any source. Since steroid receptors presumably diffuse continuously in and out of the nucleus, it is thought that this increase in nonspecific binding to DNA is what causes activated receptors to accumulate in the nucleus. Once activated by hormone, the receptor is thought also to bind with a higher affinity to the few specific sites on the chromatin of the target cell that are relevant for regulating gene transcription; however, this cannot be observed directly.

One of the most decisive experiments supporting this view of steroid receptor action involves the use of mutant cell lines selected for an altered response to cortisol. The mutant cells were derived from lymphocyte tumors, called lymphomas. Like some normal lymphocytes, lymphoma cells are killed by the addition of low concentrations of cortisol to their growth medium. The

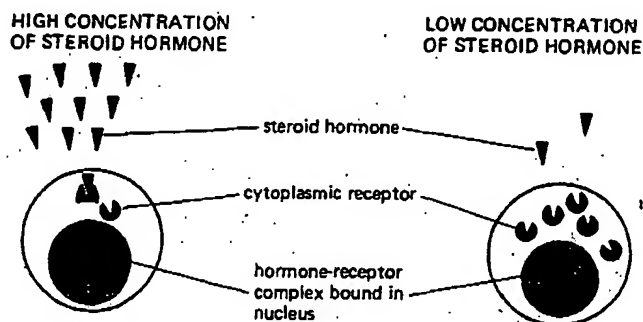
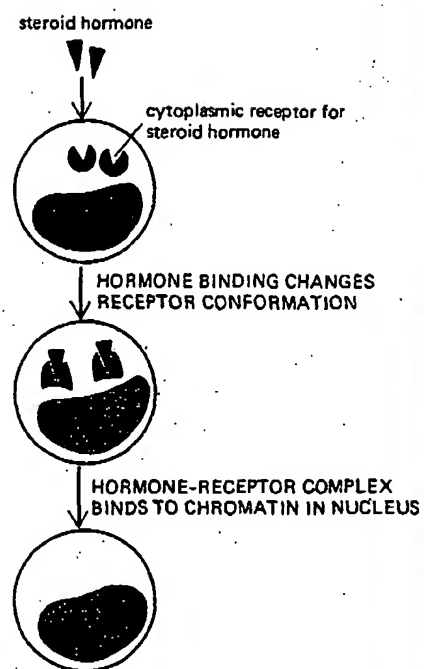


Figure 13-13 Schematic drawing showing that when steroid hormone levels are high, most of the steroid receptor proteins in a target cell are complexed to hormone and bound to chromatin in the nucleus; and when hormone levels are low, most of the receptors are free in the cytoplasm.

mutant cells, however, are resistant to the lethal effects of cortisol, and some of them are found to contain cortisol receptor proteins that fail to migrate from the cell cytoplasm to the nucleus after binding cortisol (Figure 13-14). While these mutant receptors have a normal ability to bind cortisol, they have lost most of their ability to bind to DNA. Experiments with a variety of different mutant receptor proteins show a strong correlation between the ability to bind DNA in the test tube and the extent of nuclear migration upon binding cortisol inside the cell. These experiments therefore suggest that DNA constitutes at least part of the recognition site for the receptor in the cell nucleus.

While it is generally believed that the binding of steroid-hormone-receptor complexes to specific sites in chromatin regulates specific gene transcription, it is extremely difficult to identify such sites directly. The main difficulty is that the receptors bind nonspecifically to DNA; also, the binding of only a small fraction of the cell's 10,000 hormone-receptor complexes may suffice to regulate 50 genes. Moreover, even with 10,000 receptors per cell, the receptor proteins constitute only about one part in 50,000 by weight of the total cell protein, and so only small amounts of receptor have been obtained in a highly purified form. For these reasons, it has been difficult to determine unambiguously whether steroid hormone receptors derive their specificity from the recognition of special DNA sequences, special chromosomal proteins, or both. Recently, however, recombinant DNA techniques have been used to clone a gene that is regulated by cortisol, making large amounts of specific DNA available. When this DNA was tested, purified cortisol receptors were found to bind specifically to certain DNA sequences within and around the gene. This important experiment suggests that steroid receptors recognize specific DNA sequences when they induce transcription of a gene.

Steroid Hormones Often Induce Both Primary and Secondary Responses⁷

In many cases the response to a steroid hormone takes place in two steps. The direct induction of transcription of a few specific genes is known as the *primary response*. The products of these genes may in turn, however, activate other genes and produce a delayed *secondary response*. The latter may result in a major amplification of the initial hormonal effect.

A striking example is seen in the fruit fly *Drosophila*. Within five to ten minutes of the injection of the steroid insect molting hormone *ecdysone*, six major new sites of RNA synthesis (seen as *puffs*) are induced on the giant polytene chromosomes of the salivary gland (see p. 401). After a delay, some of the proteins produced during this primary response induce an additional 100 or so sites of RNA synthesis, leading to the synthesis of a large group of proteins characteristic of the secondary response. The response is controlled by feedback through one or more of the initial proteins, which shuts off further transcription of all of the primary response genes (Figure 13-15). It is likely that similar mechanisms provide for both amplification and control in many of the responses of mammalian cells to hormones.

Steroid Hormones Regulate Different Genes in Different Target Cells⁸

The responses to steroid hormones, like hormonal responses in general, are determined as much by the nature of the target cell as by the nature of the hormone. In principle, this observation has two possible explanations. Either different types of cells have different receptors for the same hormone, or the receptors are the same but the genes activated by them are different. There is strong evidence that the latter explanation is correct.

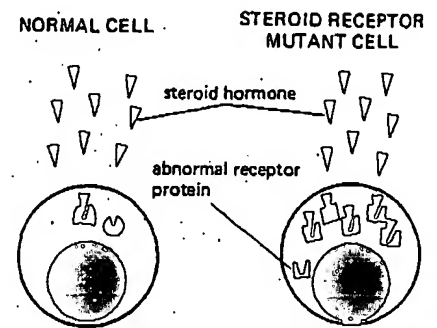
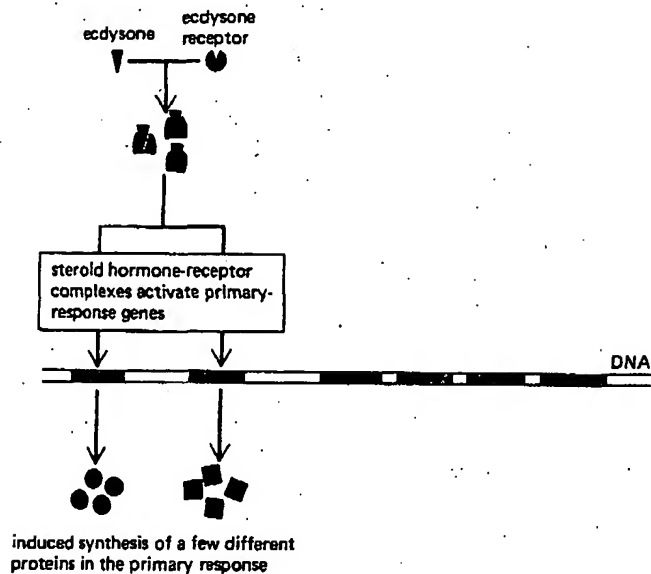
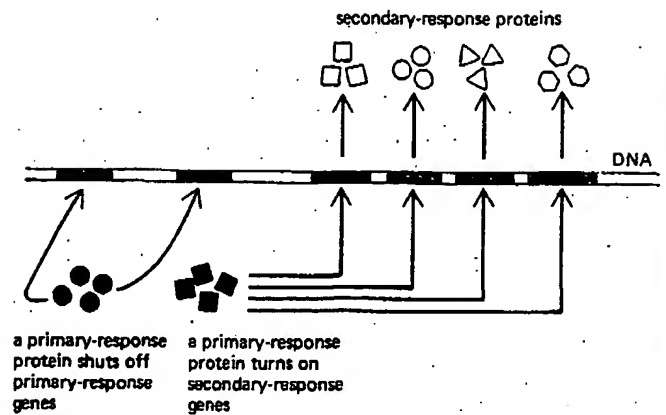


Figure 13-14 One type of steroid receptor mutant cell in which the abnormal receptors bind the hormone but then fail to localize in the nucleus.

(A) EARLY RESPONSE TO ECDYSONE



(B) DELAYED RESPONSE TO ECDYSONE



The evidence comes mainly from studies of mammalian mutants in which a particular steroid hormone receptor is abnormal. In particular, a defect in the receptor for the male hormone *testosterone* causes genetically male individuals to appear to be females, since all mammals develop along female lines unless they are exposed to testosterone during embryonic development. Mutant males have normal testosterone-secreting testes, but because their tissues have defective testosterone receptors, they cannot respond to the hormone. They therefore develop all of the secondary sexual characteristics of females, and their testes remain in the abdomen and fail to descend. This **testicular feminization syndrome** occurs in mice, rats, and cattle, as well as in man. Although only the gene coding for testosterone receptors is abnormal, all of the many different cell types in the body that are normally influenced by testosterone are affected (Figure 13-16). It follows that the same testoster-

Figure 13-15 Schematic diagram of the early primary response to ecdysone in *Drosophila* cells (A) and the delayed secondary response (B). Some of the primary-response proteins turn on secondary-response genes, while others turn off the primary-response genes. The actual number of primary- and secondary-response genes is greater than shown.

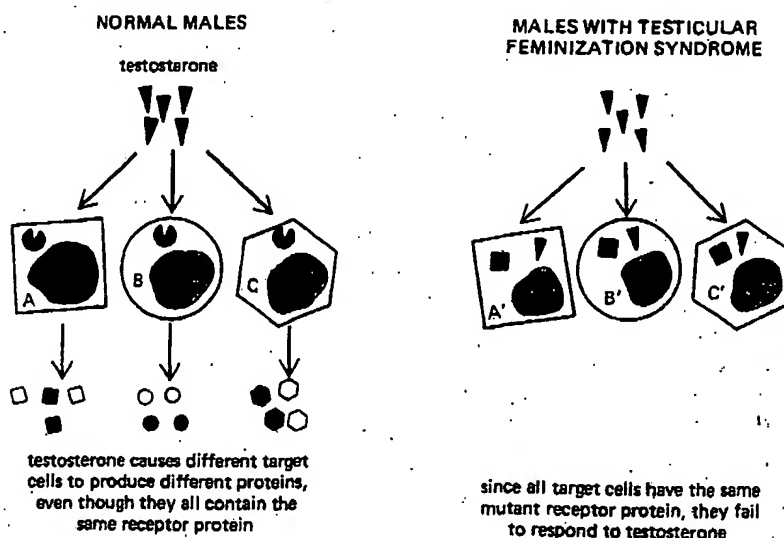


Figure 13-16 Different target cells containing the same receptor protein respond differently to testosterone. Thus a single gene defect in the testicular feminization syndrome, which leads to an abnormal testosterone receptor, results in all target cells failing to respond to testosterone.

one receptor protein must normally be present in all target cells, even though its activation regulates very different sets of genes in each type of cell.

The general principles of steroid hormone signaling are therefore clear. Each of the different steroid hormones induces a characteristic set of responses because (1) only cells designed to respond make receptors for a particular hormone and (2) the chromatin of the target cell is probably organized so that the steroid receptor activates only the appropriate genes (see Chapter 18). Since the genes available for activation are different in each type of target cell, the same hormone binding to the same steroid receptor protein has different effects on different cells.

Summary

Steroid hormones are small, hydrophobic molecules derived from cholesterol that are solubilized by binding reversibly to specific carrier proteins in the blood. Once released from their carrier proteins, they diffuse through the plasma membrane of the target cell and bind reversibly to specific steroid hormone-receptor proteins in the cytosol. By complexing to the hormone, the receptor protein acquires an affinity for DNA that causes it to accumulate in the cell nucleus. There the hormone-receptor complex binds to chromatin and regulates the transcription of a small number of genes. The products of some of these genes may, in turn, activate other genes and produce a delayed secondary response, thereby amplifying the initial effect of the hormone. Each steroid hormone is recognized by a different receptor protein, but the same receptor protein regulates different genes in different target cells. This suggests that the chromatin of each cell type is organized so as to make only the appropriate genes available for regulation by the hormone-receptor complex.

Signaling Mediated by Cell-Surface Receptors: Cyclic AMP and Calcium Ions as Second Messengers

Water-soluble signaling molecules, including all of the known neurotransmitters, protein hormones, and growth factors, bind to specific receptor proteins on the surface of the target cells they influence. These cell-surface receptors bind the signaling molecule (the ligand) with high affinity and convert this extracellular event into an intracellular signal that alters the behavior of the target cell. Since these receptors are insoluble integral membrane proteins and usually constitute less than 1% of the total protein mass of the plasma membrane, they are difficult to isolate and study.

The Use of Labeled Ligands Revolutionized the Study of Cell-Surface Receptors^a

Attempts to use radiolabeled ligands to demonstrate receptors on the surface of target cells began in the 1950s but were hampered by two problems: (1) the process of coupling the signaling molecule to a radioactive isotope (usually radioactive iodine or hydrogen) greatly reduced its functional activity; (2) most of the binding to the target cell surface was nonspecific, so that only a small fraction of the total amount of bound ligand was attached to specific receptors. Around 1970, solutions to these technical problems made it possible to demonstrate directly the presence of specific receptors on the surface of intact cells and isolated membranes.

By using ligands labeled with radioactive atoms, fluorescent dyes, or electron-dense molecules (such as ferritin), it is now possible to study the

Figure 13-17 Hypothetical scheme showing what would be needed in order for protein ligands or their cell-surface receptors to act as their own intracellular mediators: a special mechanism is required in order for the ligand or receptor (or their degradation products) to escape from an intracellular vesicle compartment into the cytosol.

numbers, distribution, and properties of specific cell-surface receptors as well as to follow the fate of the ligand-receptor complexes after binding. It has been shown that the number of receptors for a specific ligand can vary from 500 to more than 100,000 per cell and that the initial receptor distribution can be either diffuse or localized to specific regions of the plasma membrane.

Protein Hormones and Growth Factors Are Ingested by Receptor-mediated Endocytosis¹⁰

Experiments with labeled ligands have demonstrated that many protein signaling molecules enter target cells by receptor-mediated endocytosis (see p. 309). For example, insulin binds to receptor proteins diffusely distributed on the surface of fibroblasts; within minutes the insulin-receptor complexes cluster in coated pits and are ingested in endocytotic vesicles. It is therefore conceivable that such protein signaling molecules (or their degradation products) act directly within the cell, much as steroid and thyroid hormones do. However, it must be remembered that receptor-mediated endocytosis usually results in the transfer of extracellular molecules to lysosomes (p. 309). In order to enter the cytosol, these hydrophilic molecules would require some special mechanism for escaping from either the endocytotic vesicle or the lysosome (Figure 13-17).

It is possible to show by a direct experiment that at least some signaling molecules do not have to enter cells in order to influence them. For example, the effects of insulin can be exactly mimicked by specific antibodies that bind to insulin receptors on the surface of target cells. Therefore, although insulin is normally endocytosed by target cells, the ingested hormone cannot itself be the intracellular signal. Similarly, while *thyroid-stimulating hormone* (TSH) normally activates thyroid cells to synthesize and secrete thyroid hormone, antibodies binding to the TSH receptors on the surface of thyroid cells can be just as effective (Figure 13-18). In fact, such antibodies are the usual cause of hyperthyroid disease in man, a condition in which too much thyroid hor-

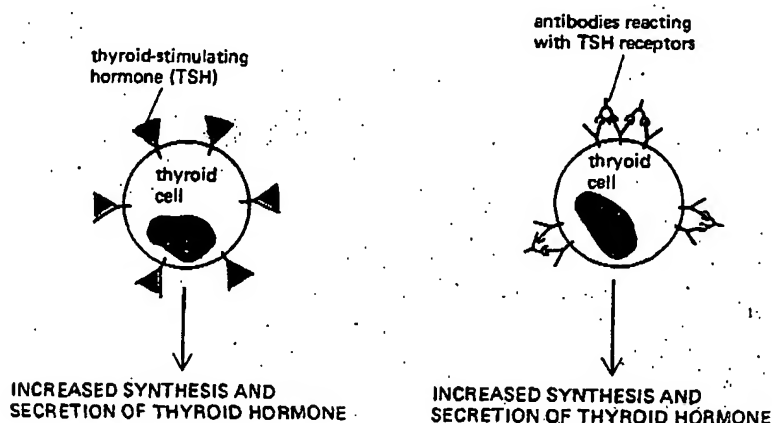
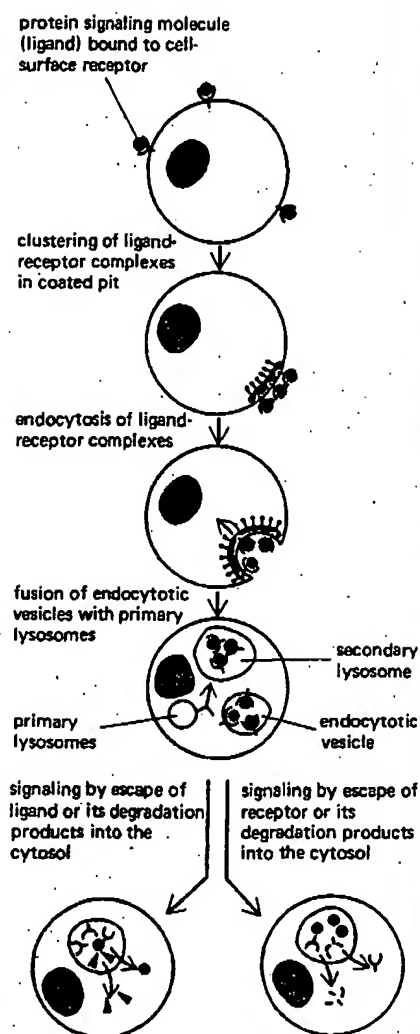


Figure 13-18 Antibodies directed against cell-surface receptors for thyroid-stimulating hormone (TSH) mimic the effects of TSH binding to these receptors. This suggests that TSH is not required for the signaling process and therefore probably does not act as its own intracellular mediator.

none is produced. While these observations demonstrate that insulin and TSH do not have to enter the cell in order to act, they do not exclude the possibility that the cell-surface receptors for these hormones may act as intracellular signals, since the receptors are normally endocytosed along with either the bound hormone or the antibody (Figure 13-17).

Many hydrophilic signaling molecules induce responses that occur much too rapidly to involve receptor-mediated endocytosis. Mast cells secrete histamine within seconds of ligand binding to surface receptors, while the responses to some neurotransmitters occur in milliseconds. In these cases the receptors must instead act as transmembrane transducers to generate new intracellular signals.

Cell-Surface Receptors Act as Transducers by Regulating Enzymes or Ion Channels in the Plasma Membrane¹¹

The great majority of cell-surface receptors that bind hydrophilic signaling molecules are thought to undergo a conformational change when they bind to a ligand at the cell exterior. This change leads to the generation of an intracellular signal that alters the behavior of the target cell. The intracellular signaling molecules are often referred to as *second messengers*, the *first messengers* being the extracellular ligands themselves.

There are two general ways in which cell-surface receptors are known to generate intracellular signals. One is by activating or inactivating a plasma-membrane-bound enzyme. In some cases this enzyme catalyzes the production of a soluble intracellular mediator; the change in the intracellular concentration of the mediator then serves as the signal. An important enzyme that acts in this way is *adenylate cyclase*, which catalyzes the synthesis of *cyclic AMP (cAMP)* from ATP on the cytoplasmic side of the plasma membrane (Figure 13-19). In other cases the enzyme activated by an extracellular ligand directly causes the phosphorylation of cellular proteins. For example, *epidermal growth factor (EGF)* stimulates epidermal cells and a variety of other cell types to divide by binding to receptor proteins on the cell surface. The receptors are (or are closely associated with) protein kinases that are activated by the binding of EGF to transfer a phosphate from ATP to a tyrosine residue on specific cellular proteins, including the receptor protein itself and other plasma membrane proteins as well as some cytosolic proteins.

Alternatively, cell-surface receptors may open or close gated ion channels in the plasma membrane. This generates a signal in either of two ways: (1) it causes a small and transient flux of ions that briefly changes the voltage across the plasma membrane, or (2) it causes a major influx of ions into the cytosol, which in turn initiates an intracellular response. The first mechanism operates mainly in electrically active cells such as neurons and muscle cells. For example, most neurotransmitters regulate the membrane potential of the postsynaptic target cell by opening or closing ion channels in the target cell plasma membrane: a decrease in the membrane potential below a certain threshold level triggers an explosive depolarization of the membrane (an *action potential*), which rapidly spreads to the rest of the target cell membrane. These changes in membrane potential are not accompanied by an appreciable change in ion concentration in the cytosol, so that the initial signal localized at the postsynaptic plasma membrane is not converted into a truly intracellular signal until the action potential reaches the nerve terminal. Then, because the plasma membrane of the nerve terminal contains voltage-gated Ca^{2+} channels that are transiently opened when the membrane is depolarized by the action potential, Ca^{2+} enters the terminal down its very steep electrochemical gradient and acts as a second messenger to initiate neurotransmitter secretion (see Chapter 18).

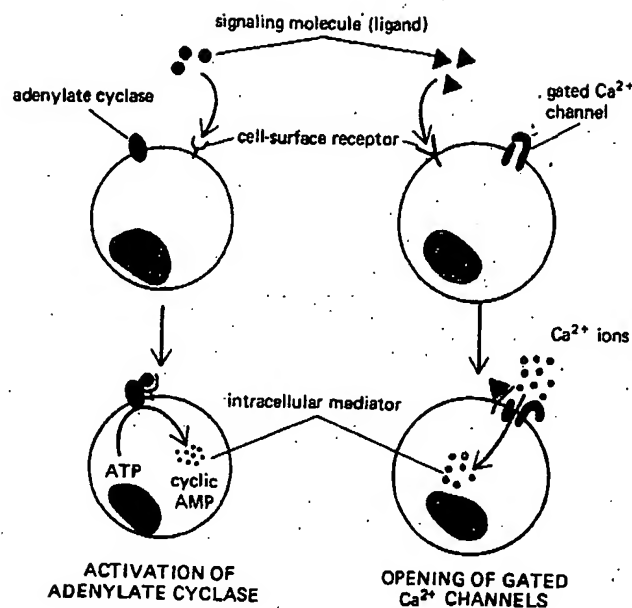


Figure 13-19 Two common mechanisms by which cell-surface receptors generate intracellular signals: (1) the activation of membrane-bound adenylate cyclase molecules increases the intracellular concentration of cyclic AMP; and (2) the opening of membrane-bound, gated Ca^{2+} channels allows Ca^{2+} to enter the cell. For simplicity, the receptors are shown to interact directly with the cyclase molecules or ion channels following ligand binding. In fact, other membrane proteins mediate the coupling of receptors to the cyclase molecules and possibly also to Ca^{2+} channels.

Many animal cells that are not electrically active have cell-surface receptors that are functionally linked to Ca^{2+} channels in the plasma membrane: ligand binding activates these receptors, thereby opening the channels and allowing Ca^{2+} to enter the cytosol, where it then functions as a second messenger (Figure 13-19).

Cyclic AMP Is a Ubiquitous Intracellular Mediator¹²

Cyclic AMP (Figure 13-20) regulates intracellular reactions in all procaryotic and nucleated animal cells that have been studied to date. Although it serves as an important intracellular signaling molecule, it seems not to be required for cell survival or division, since some mutant eucaryotic cell lines that make no detectable cyclic AMP grow normally in culture.

The identification of cyclic AMP as a common intracellular mediator for various hormones was a major advance. The first evidence for such a soluble intracellular mediator came from studies on the effects of the hormone *epinephrine* on glycogen metabolism in liver cells. It was found that epinephrine causes the activation of the enzyme *glycogen phosphorylase*, which catalyzes

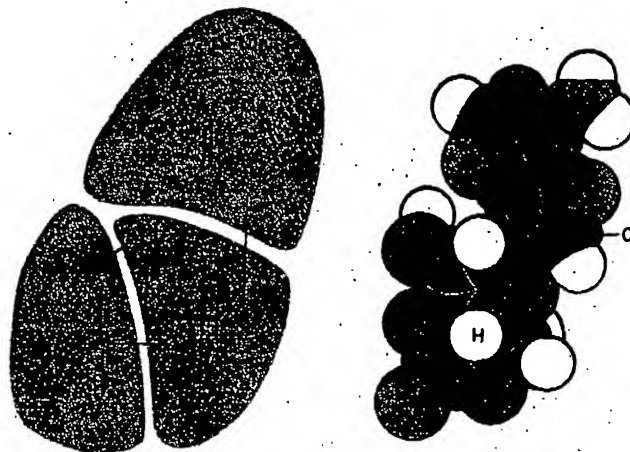


Figure 13-20 Cyclic AMP shown as a formula and as a space-filling model. (C, H, N, O, and P indicate carbon, hydrogen, nitrogen, oxygen, and phosphorus atoms, respectively.)

the breakdown of glycogen. It was then possible to show that treating isolated liver cell membranes with epinephrine (in the presence of ATP) induced the production of a small, heat-labile mediator that could substitute for the hormone and activate the phosphorylase present in a membrane-free extract of liver cells. The mediator was identified in 1959 as cyclic AMP.

For cyclic AMP to function as an intracellular mediator, its intracellular concentration (normally $\leq 10^{-6}$ M) must be tightly controlled and able to change rapidly in response to extracellular signals: upon hormonal stimulation, cyclic AMP levels can change by fivefold in seconds. Cyclic AMP is synthesized from ATP by the plasma-membrane-bound enzyme *adenylate cyclase*, but it is also rapidly destroyed in cells by one or more specific enzymes called *phosphodiesterases*, which hydrolyze cyclic AMP to adenosine 5'-monophosphate (5'-AMP) (Figure 13-21). Cell-surface receptors for which cyclic AMP is the intracellular messenger act by altering (usually stimulating) the activity of *adenylate cyclase* rather than by altering *phosphodiesterase* activity. However, the continuous rapid breakdown or removal of any intracellular mediator is also required in order to obtain either a rapid increase or a rapid decrease in its concentration, as explained below (see p. 750).

Receptor and Adenylate Cyclase Molecules Are Separate Proteins That Functionally Interact in the Plasma Membrane¹³

Many hormones and local chemical mediators work by activating *adenylate cyclase*. Several examples, and the effects they produce, are listed in Table 13-2. Just as the same steroid hormone produces different effects in different target cells, so different target cells respond very differently to changes in their intracellular cyclic AMP levels.

Since each type of animal cell responds to an increase in cyclic AMP in a characteristic way, any ligand that activates *adenylate cyclase* in a given target cell usually produces the same effect. For example, at least four different hormones activate *adenylate cyclase* in fat cells, and all of them stimulate the breakdown of triglyceride (the storage form of fat) to fatty acids (Table 13-2). This can be explained in two ways: either each different type of receptor is tightly linked to its own *adenylate cyclase* molecule in the plasma membrane, or the different receptors share a common pool of *adenylate cyclase* molecules. That the latter is the case is suggested by receptor "transplantation" experiments. For example, epinephrine receptors isolated from detergent-solubilized plasma membranes can be transplanted to the plasma membrane of

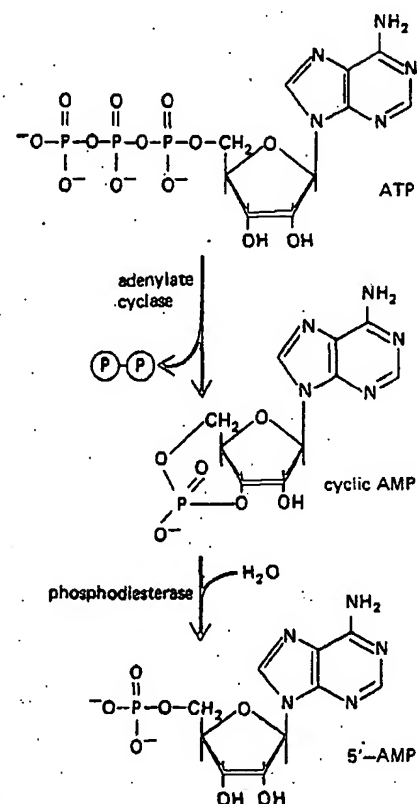


Figure 13-21 The synthesis and degradation of cyclic AMP.

Table 13-2 Some Hormone-induced Effects in Different Target Cells Mediated by Cyclic AMP

Target Tissue	Hormone	Major Response
Thyroid	thyroid-stimulating hormone	thyroxine secretion
Adrenal cortex	adrenocorticotrophic hormone (ACTH)	cortisol secretion
Ovary	luteinizing hormone	progesterone secretion
Muscle, Liver	epinephrine	glycogen breakdown
Bone	parathormone	bone resorption
Heart	epinephrine	increase in heart rate
Kidney	vasopressin	water resorption
Fat	epinephrine, ACTH, glucagon, thyroid-stimulating hormone	triglyceride breakdown

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